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# THE INFLUENCE OF ANTIGEN DILUTION ON THE WASSERMANN REACTION

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In a previous report<sup>1</sup> it was shown that higher dilutions of antigens gave stronger positive results in the Wassermann reaction than did lower dilutions of the same antigen. At the present time the results obtained in a more detailed study on the influence of antigen turbidity and antigen dilution will be reported.

## TECHNIC

The human serums were heated to about 56 C. for 30 minutes and were glycerolated. All ingredients were so prepared that the test doses were uniform. The test dose of each prepared ingredient was 0.2 cc which gave a total quantity of 1 cc for each test tube.

As complement serum the mixed serums of five guinea-pigs were used in dilutions of 1:5, 1:10 and 1:20.

Three different antigens were used, alcoholic extract of human heart (A. E. H. H.), alcoholic extract of beef heart (A. E. B. H.) and alcoholic extract of rabbit heart (A. E. R. H.). Of each antigen six different portions, A, B, C, D, E and F were prepared. For portion A, 0.1 cc of alcoholic extract was put into a test tube, 0.05 of salt solution was added, the mixture shaken, another 0.05 cc of salt solution was added, the mixture was again shaken and was allowed to stand for about 5 minutes, when quantities of 0.1 cc of physiologic salt solution were added until the mixture amounted to 1 cc, the mixture having been well shaken after each addition of salt solution. In any further dilution of the antigen solution the salt solution was added in quantities of 0.2 cc. Portion A of the antigen solution was used in dilutions of 1:25, 1:50, 1:100, 1:200, and 1:400. For portion B, 0.1 cc of alcoholic extract was put into a test tube, 0.05 cc of salt solution was added, the mixture was shaken, another 0.05 cc of salt solution was added, the mixture was again shaken and was diluted up to 1 cc by the addition of 0.1 cc quantities of salt solution. In further diluting the antigen solution the salt solution was added in quantities of 0.2 cc. Portion B was used in dilutions of 1:25, 1:50, 1:100, 1:200 and 1:400. For portion C 0.1 cc of alcoholic extract was put into a test tube, 0.1 cc of salt solution was added, the mixture was shaken, the dilution was continued by the addition of 0.1 cc quantities of salt solution until the mixture amounted to 1 cc, after which quantities of 0.2 cc were used. Like portions A and B portion C was used in dilutions of 1:25, 1:50, 1:100, 1:200 and 1:400. For portion D 0.1 cc of alcoholic extract was put into a test tube and was diluted by adding 0.2 cc quantities of salt solution. Portion D, like the previous portions, was used in dilutions of 1:25, 1:50, 1:100, 1:200 and 1:400. For portion E 0.1 cc of alcoholic extract was put

into a test tube and was diluted by adding 0.3 c c quantities of salt solution. Portion E was used in dilutions of 1:25, 1:50, 1:100, 1:200 and 1:400. In portion F 0.1 c c of alcoholic extract was diluted by adding 0.4 c c quantities of salt solution. Portion F was used in dilutions of 1:25, 1:50, 1:100, 1:200 and 1:400. Whenever the quantity of salt solution necessary for a certain dilution was not a multiple of the quantities added the proper dilution was obtained by adding the necessary fraction of the regular quantity as,  $1 + 0.2 + 0.2 + 0.2 + 0.2 + 0.2 + 0.2 + 0.2 + 1 = 2.5$ .

The antihuman hemolytic system was used. Hemolytic amboceptor was used in doses of 1 unit per test tube and the blood corpuscles were used in the form of a 2.5% suspension in salt solution.

Each human serum was tested with each of the 30 different antigen solutions at the same time; complement, blood corpuscles and hemolytic amboceptor having been the same any difference in the results must have been due to difference in the antigen solution. First incubation was in the water-bath at 1 C. for 5 hours followed by 30 minutes in the incubator at 37 C. and second incubation was in the incubator at 37 C. for 1 hour. The results were read and recorded about 2 hours after the sensitized blood corpuscles had been added.

TEST 1

Human serums 1 and 2 from syphilitics under treatment and 3 and 4 from nonsyphilitics were each tested with the 30 different solutions of alcoholic extract of human heart.

TABLE 1

SOLUTIONS OF ALCOHOLIC EXTRACT OF HUMAN HEART OF DIFFERENT DEGREES OF TURBIDITY  
TESTED IN DILUTIONS VARYING FROM 1:25 TO 1:400

Number of Serum	Kind of Antigen	Portion	Dilution of Antigen	Readings*									Results
				Antigen Tubes			Control Tubes						
				1	2	3	1'	2'	3'				
1	A. E. H. H.	A	1:25	+	±	0	+	+	±	Strongly positive, 3+ Moderately positive, 2+ Moderately positive, 2+ Weakly positive, 1+ Faintly positive, ±			
			1:50	+	+	0	+	+	±				
			1:100	+	+	0	+	+	±				
			1:200	+	+	tr	+	+	±				
			1:400	+	+	±?	+	+	±				
		B	1:25	+	0	0	+	+	±	Strongly positive, 5+ Strongly positive, 5+ Strongly positive, 4+ Moderately positive, 2+ Weakly positiv,e 1+			
			1:50	+	0	0	+	+	±				
			1:100	+	tr	0	+	+	±				
			1:200	+	+	0	+	+	±				
			1:400	+	+	tr	+	+	±				
		C	1:25	+	0	0	+	+	±	Strongly positive, 5+ Strongly positive, 6+ Strongly positive, 5+ Strongly positive, 3+ Moderately positive, 2+			
			1:50	±	0	0	+	+	±				
			1:100	+	0	0	+	+	±				
			1:200	+	±	0	+	+	±				
			1:400	+	+	0	+	+	±				
		D	1:25	+	+	0	+	+	±	Moderately positive, 2+ Strongly positive, 3+ Strongly positive, 4+ Strongly positive, 4+ Moderately positive, 2+			
			1:50	+	±	0	+	+	±				
			1:100	+	tr	0	+	+	±				
			1:200	+	tr	0	+	+	±				
			1:400	+	+	0	+	+	±				

\* Explanation: In all tables + means complete hemolysis; ±, hemolysis between 50 and 100%; tr (trace) hemolysis up to 50%; 0, no hemolysis.

Serums 3 and 4, tested exactly in the same way as serums 1 and 2, gave uniformly negative results.

TABLE 1—Continued  
SOLUTIONS OF ALCOHOLIC EXTRACT OF HUMAN HEART OF DIFFERENT DEGREES OF TURBIDITY  
TESTED IN DILUTIONS VARYING FROM 1:25 TO 1:400

Number of Serum	Kind of Antigen	Por- tion	Dilution of Antigen	Readings						Results
				Antigen Tubes			Control Tubes			
				1	2	3	1'	2'	3'	
2	A. E. H. H.	E	1:25	+	+	tr	+	+	±	Weakly positive, 1+ Moderately positive, 2+ Strongly positive, 3+ Strongly positive, 3+ Moderately positive, 2+
			1:50	+	+	0	+	+	±	
			1:100	+	±	0	+	+	±	
			1:200	+	±	0	+	+	±	
			1:400	+	+	0	+	+	±	
		F	1:25	+	+	±?	+	+	±	Faintly positive, ± Weakly positive, 1+ Moderately positive, 2+ Moderately positive, 2+ Moderately positive, 2+
			1:50	+	+	tr	+	+	±	
			1:100	+	+	0	+	+	±	
			1:200	+	+	0	+	+	±	
			1:400	+	+	0	+	+	±	
		A	1:25	+	tr	0	+	+	±	Strongly positive, 4+ Strongly positive, 4+ Strongly positive, 3+ Weakly positive, 1+ Faintly positive, ±
			1:50	+	tr	0	+	+	±	
			1:100	+	±	0	+	+	±	
			1:200	+	+	tr	+	+	±	
			1:400	+	+	±?	+	+	±	
		B	1:25	+	±	0	+	+	±	Strongly positive, 3+ Strongly positive, 4+ Strongly positive, 3+ Moderately positive, 2+ Weakly positive, 1+
			1:50	+	tr	0	+	+	±	
			1:100	+	±	0	+	+	±	
			1:200	+	+	0	+	+	±	
			1:400	+	+	tr	+	+	±	
		C	1:25	+	±	0	+	+	±	Strongly positive, 3+ Strongly positive, 5+ Strongly positive, 5+ Strongly positive, 3+ Moderately positive, 2+
			1:50	+	0	0	+	+	±	
			1:100	+	0	0	+	+	±	
			1:200	+	±	0	+	+	±	
			1:400	+	+	0	+	+	±	
		D	1:25	+	+	±	+	+	±	Negative, — Weakly positive, 1+ Moderately positive, 2+ Strongly positive, 3+ Moderately positive, 2+
			1:50	+	+	tr	+	+	±	
			1:100	+	+	0	+	+	±	
			1:200	+	±	0	+	+	±	
			1:400	+	+	0	+	+	±	
		E	1:25	+	+	±	+	+	±	Negative, — Faintly positive, ± Weakly positive, 1+ Moderately positive, 2+ Moderately positive, 2+
			1:50	+	+	±?	+	+	±	
			1:100	+	+	tr	+	+	±	
			1:200	+	+	0	+	+	±	
			1:400	+	+	0	+	+	±	
		F	1:25	+	+	±	+	+	±	Negative, — Negative, — Faintly positive, ± Moderately positive, 2+ Moderately positive, 2+
			1:50	+	+	±	+	+	±	
			1:100	+	+	±?	+	+	±	
			1:200	+	+	0	+	+	±	
			1:400	+	+	0	+	+	±	

The results obtained by testing the 30 different solutions of the same alcoholic extract of human heart are shown in table 1. Portion C gave the strongest positive results with the syphilitic serums and the strongest positive results were obtained with the dilution of 1:50. In portions A and B the stronger results were obtained with lower dilutions of antigen and with portions D, E and F the higher dilutions, such as 1:100 and 1:200, gave stronger positive results than did the lower dilutions. The serums from nonsyphilitic persons gave negative results throughout.

## TEST 2

With serums 5 and 6 from known syphilitics under treatment and 7 and 8 from nonsyphilitic persons each of the six different portions, A, B, C, D, E and F of alcoholic extract of beef heart was tested in 5 different dilutions, dilutions of 1:25, 1:50, 1:100, 1:200 and 1:400.

TABLE 2  
SOLUTIONS OF ALCOHOLIC EXTRACT OF BEEF HEART OF DIFFERENT DEGREES OF TURBIDITY  
TESTED IN DILUTIONS VARYING FROM 1:25 TO 1:400

Number of Serum	Kind of Antigen	Portion	Dilution of Antigen	Readings						Results	
				Antigen Tubes			Control Tubes				
				1	2	3	1'	2'	3'		
5	A. E. B. H.	A	1:25	±	0	0	+	+	±	Strongly positive,	6+
			1:50	tr	0	0	+	+	±	Strongly positive,	8+
			1:100	±	0	0	+	+	±	Strongly positive,	6+
			1:200	+	±	0	+	+	±	Strongly positive,	3+
			1:400	+	+	0	+	+	±	Moderately positive,	2+
		B	1:25	+	0	0	+	+	±	Strongly positive,	5+
			1:50	tr	0	0	+	+	±	Strongly positive,	8+
			1:100	+	0	0	+	+	±	Strongly positive,	5+
			1:200	+	0	0	+	+	±	Strongly positive,	5+
			1:400	+	±	0	+	+	±	Strongly positive,	3+
		C	1:25	+	tr	0	+	+	±	Strongly positive,	4+
			1:50	+	0	0	+	+	±	Strongly positive,	5+
			1:100	±	0	0	+	+	±	Strongly positive,	6+
			1:200	+	tr	0	+	+	±	Strongly positive,	4+
			1:400	+	tr	0	+	+	±	Strongly positive,	4+
		D	1:25	+	+	tr	+	+	±	Weakly positive,	1+
			1:50	+	+	tr	+	+	±	Weakly positive,	1+
			1:100	+	+	0	+	+	±	Moderately positive,	2+
			1:200	+	±	0	+	+	±	Strongly positive,	3+
			1:400	+	±	0	+	+	±	Strongly positive,	3+
		E	1:25	+	+	tr	+	+	±	Weakly positive,	1+
			1:50	+	+	tr	+	+	±	Weakly positive,	1+
			1:100	+	+	0	+	+	±	Moderately positive,	2+
			1:200	+	±	0	+	+	±	Strongly positive,	3+
1:400	+		±	0	+	+	±	Strongly positive,	3+		
F	1:25	+	+	tr	+	+	±	Weakly positive,	1+		
	1:50	+	+	tr	+	+	±	Weakly positive,	1+		
	1:100	+	+	tr	+	+	±	Weakly positive,	1+		
	1:200	+	+	0	+	+	±	Moderately positive,	2+		
	1:400	+	+	0	+	+	±	Moderately positive,	2+		
6	A. E. B. H.	A	1:25	+	tr	0	+	+	±	Strongly positive,	4+
			1:50	+	0	0	+	+	±	Strongly positive,	5+
			1:100	+	tr	0	+	+	±	Strongly positive,	4+
			1:200	+	+	0	+	+	±	Moderately positive,	2+
			1:400	+	+	tr	+	+	±	Weakly positive,	1+
		B	1:25	+	tr	0	+	+	±	Strongly positive,	4+
			1:50	+	0	0	+	+	±	Strongly positive,	5+
			1:100	+	0	0	+	+	±	Strongly positive,	5+
			1:200	+	±	0	+	+	±	Strongly positive,	3+
			1:400	+	+	0	+	+	±	Moderately positive,	2+
		C	1:25	+	+	tr	+	+	±	Weakly positive,	1+
			1:50	+	+	0	+	+	±	Moderately positive,	2+
			1:100	+	±	0	+	+	±	Strongly positive,	3+
			1:200	+	±	0	+	+	±	Strongly positive,	3+
			1:400	+	+	0	+	+	±	Moderately positive,	2+

Serums 7 and 8, nonsyphilitic, tested in the same way as serums 5 and 6, gave uniformly negative results.

TABLE 2—Continued  
SOLUTIONS OF ALCOHOLIC EXTRACT OF BEEF HEART OF DIFFERENT DEGREES OF TURBIDITY  
TESTED IN DILUTIONS VARYING FROM 1:25 TO 1:400

Number of Serum	Kind of Antigen	Portion	Dilution of Antigen	Readings						Results
				Antigen Tubes			Control Tubes			
				1	2	3	1'	2'	3'	
	D	1:25	+	+	± <sup>?</sup>	+	+	±	Faintly positive,	±
		1:50	+	+	± <sup>?</sup>	+	+	±	Faintly positive,	±
		1:100	+	+	tr	+	+	±	Weakly positive,	1+
		1:200	+	+	0	+	+	±	Moderately positive,	2+
		1:400	+	+	0	+	+	±	Moderately positive,	2+
	E	1:25	+	+	±	+	+	±	Negative,	—
		1:50	+	+	± <sup>?</sup>	+	+	±	Faintly positive,	±
		1:100	+	+	± <sup>?</sup>	+	+	±	Faintly positive,	±
		1:200	+	+	tr	+	+	±	Weakly positive,	1+
		1:400	+	+	0	+	+	±	Moderately positive,	2+
	F	1:25	+	+	±	+	+	±	Negative,	—
		1:50	+	+	±	+	+	±	Negative,	—
		1:100	+	+	± <sup>?</sup>	+	+	±	Faintly positive,	±
		1:200	+	+	tr	+	+	±	Weakly positive,	1+
		1:400	+	+	0	+	+	±	Moderately positive,	2+

Table 2 shows the results obtained with the 30 different solutions of alcoholic extract of beef heart. Portions A and B gave the strongest positive results. In portions A and B the dilution of 1:50 gave the strongest positive results, in portion C 1:100 gave the strongest positive results and of portions D, E and F the dilutions of 1:200 and 1:400 gave stronger positive results than did dilutions of 1:25, 1:50 and 1:100. Although dilutions of 1:200 and 1:400 of portions D, E and F gave stronger positive results than did the lower dilutions of the same portions these results were greatly inferior to those obtained with portions A and B in dilutions of 1:50. With the serums from nonsyphilitic persons negative results were obtained throughout.

### TEST 3

The 30 different solutions of alcoholic extract of rabbit heart were tested on two serums from known syphilitics and two from nonsyphilitic persons, serums Nos. 9, 10, 11 and 12.

TABLE 3  
SOLUTIONS OF ALCOHOLIC EXTRACT OF RABBIT HEART OF DIFFERENT DEGREES OF TURBIDITY  
TESTED IN DILUTIONS VARYING FROM 1:25 TO 1:400

Number of Serum	Kind of Antigen	Portion	Dilution of Antigen	Readings						Results
				Antigen Tubes			Control Tubes			
				1	2	3	1'	2'	3'	
9	A. E. R. H.	A	1:25	+	+	tr	+	+	±	Weakly positive, 1+ Moderately positive, 2+ Strongly positive, 3+ Moderately positive, 2+ Weakly positive, 1+
			1:50	+	+	0	+	+	±	
			1:100	+	±	0	+	+	±	
			1:200	+	+	0	+	+	±	
			1:400	+	+	tr	+	+	±	

Serums 11 and 12, nonsyphilitic, tested in the same way as serums 9 and 10, gave uniformly negative results.

TABLE 3—Continued

SOLUTIONS OF ALCOHOLIC EXTRACT OF RABBIT HEART OF DIFFERENT DEGREES OF TURBIDITY  
TESTED IN DILUTIONS VARYING FROM 1:25 TO 1:400

Number of Serum	Kind of Antigen	Por- tion	Dilution of Antigen	Readings						Results	
				Antigen Tubes			Control Tubes				
				1	2	3	1'	2'	3'		
10	A. E. R. H.	B	1:25	+	+	0	+	+	±	Moderately positive, 2+	
			1:50	+	±	0	+	+	±	Strongly positive, 3+	
			1:100	+	±	0	+	+	±	Strongly positive, 3+	
			1:200	+	+	0	+	+	±	Moderately positive, 2+	
			1:400	+	+	tr	+	+	±	Weakly positive, 1+	
		C	1:25	+	+	tr	+	+	±	Weakly positive, 1+	
			1:50	+	±	0	+	+	±	Strongly positive, 3+	
			1:100	+	tr	0	+	+	±	Strongly positive, 4+	
			1:200	+	±	0	+	+	±	Strongly positive, 3+	
			1:400	+	+	0	+	+	±	Moderately positive, 2+	
		D	1:25	+	+	±	+	+	±	Negative, —	
			1:50	+	+	±?	+	+	±	Faintly positive, ±	
			1:100	+	+	tr	+	+	±	Weakly positive, 1+	
			1:200	+	+	tr	+	+	±	Weakly positive, 1+	
			1:400	+	+	0	+	+	±	Moderately positive, 2+	
		E	1:25	+	+	±	+	+	±	Negative, —	
			1:50	+	+	±	+	+	±	Negative, —	
			1:100	+	+	±?	+	+	±	Faintly positive, ±	
			1:200	+	+	tr	+	+	±	Weakly positive, 1+	
			1:400	+	+	tr	+	+	±	Weakly positive, 1+	
		F	1:25	+	+	±	+	+	±	Negative, —	
			1:50	+	+	±	+	+	±	Negative, —	
			1:100	+	+	±	+	+	±	Negative, —	
			1:200	+	+	tr	+	+	±	Weakly positive, 1+	
			1:400	+	+	tr	+	+	±	Weakly positive, 1+	
		A	A	1:25	+	+	0	+	+	±	Moderately positive, 2+
				1:50	+	±	0	+	+	±	Strongly positive, 3+
				1:100	+	+	0	+	+	±	Moderately positive, 2+
				1:200	+	+	0	+	+	±	Moderately positive, 2+
				1:400	+	+	tr	+	+	±	Weakly positive, 1+
			B	1:25	+	+	0	+	+	±	Moderately positive, 2+
				1:50	+	+	0	+	+	±	Moderately positive, 2+
				1:100	+	+	0	+	+	±	Moderately positive, 2+
				1:200	+	+	0	+	+	±	Moderately positive, 2+
				1:400	+	+	tr	+	+	±	Weakly positive, 1+
			C	1:25	+	+	tr	+	+	±	Weakly positive, 1+
				1:50	+	±	0	+	+	±	Strongly positive, 3+
				1:100	+	tr	0	+	+	±	Strongly positive, 4+
				1:200	+	±	0	+	+	±	Strongly positive, 3+
				1:400	+	+	0	+	+	±	Moderately positive, 2+
			D	1:25	+	+	±?	+	+	±	Faintly positive, ±
				1:50	+	+	±?	+	+	±	Faintly positive, ±
				1:100	+	+	tr	+	+	±	Weakly positive, 1+
				1:200	+	+	0	+	+	±	Moderately positive, 2+
				1:400	+	+	0	+	+	±	Moderately positive, 2+
		E	1:25	+	+	±	+	+	±	Negative, —	
			1:50	+	+	±?	+	+	±	Faintly positive, ±	
			1:100	+	+	±?	+	+	±	Faintly positive, ±	
			1:200	+	+	tr	+	+	±	Weakly positive, 1+	
			1:400	+	+	tr	+	+	±	Weakly positive, 1+	
		F	1:25	+	+	±	+	+	±	Negative, —	
			1:50	+	+	±	+	+	±	Negative, —	
			1:100	+	+	±	+	+	±	Negative, —	
			1:200	+	+	±?	+	+	±	Faintly positive, ±	
			1:400	+	+	tr	+	+	±	Weakly positive, 1+	

Table 3 shows the results obtained with the 30 different solutions of alcoholic extract of rabbit heart on two serums giving positive results and two serums giving negative results. Portion C gave the strongest positive results, and the strongest results of all was obtained with portion C diluted 1:100. Of portions A and B, the strongest results were obtained with dilutions of 1:50 and 1:100, while portions D, E and F gave their strongest results in the higher dilutions. On the serums from the nonsyphilitic persons negative results were obtained throughout.

#### SUMMARY

The turbidity of the antigen solution and the dilution of the antigen solution greatly influenced the results obtained with the Wassermann reaction with serums from syphilitic persons.

For each antigen there seems to be an optimum turbidity and an optimum dilution. Antigen diluted too slowly or too rapidly lost in antigenic value.

The optimum dose of antigen would be the proper dose to use in the Wassermann reaction. The optimum dilution of antigen may be somewhat higher than is commonly used; alcoholic extract of human heart gave the strongest positive results in a dilution of 1:50 and alcoholic extract of beef heart and of rabbit heart gave the strongest positive results in dilutions of 1:100.

When the antigen was diluted too slowly the optimum dilution of that particular solution was low, and when the antigen was diluted too rapidly the optimum dilution of that particular solution was high; but neither was equal in antigenic value to the optimum dilution of the optimum turbidity.

With serums from nonsyphilitic persons the results were uniformly negative.